Supplementary Information for

Temperature-mediated Molecular Ladder Self-assembly Employing Diels-Alder Cycloaddition

Samuel C. Leguizamon,[a] Abdulla F. Alqubati,[a] and Timothy F. Scott*[,b,c]

[a] Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109, USA.

[b] Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia.

[c] Department of Materials Science and Engineering, Monash University, Clayton, VIC 3800, Australia.

General experimental procedures

^{1}H NMR spectra of the monomers were collected using a Bruker Avance 400 NMR spectrometer. Chemical shifts were measured in δ (ppm) relative to residual solvent (CD_3CN = 1.94, D_2O = 4.79, and $CDCl_3$ = 7.26). Electrospray ionization (ESI) mass spectra were recorded using an Agilent UPLC 1290 Q-TOF 6500 series spectrometer in positive ion mode. Reverse phase high performance liquid chromatography (RP-HPLC) was performed using both a preparative reversed phase Phenomenex Luna C18(2) column with a linear gradient of water and acetonitrile as the eluent at 30°C as well as an analytical scale column. Gel permeation chromatography (GPC) was performed using three Phenogel GPC/SEC columns (length 300 mm × diameter 7.8 mm, pore sizes of 500, 100, and 50 Å) in series, 92:6:2 (v/v/v) $CHCl_3$:MeOH:Et_3N as the eluent at room temperature. The RP-HPLC and GPC systems were equipped with a dual-parallel Shimadzu LC-20AD HPLC pump, a Shimadzu FRC-10A fraction collector, and monitored using a Shimadzu Prominence UV-Vis detector at 214, 254, or 313 nm. Unless otherwise noted, all reagents and materials were purchased from Sigma Aldrich, AK Scientific, Oakwood Products, and TCI America.

Monomer Synthesis

Synthesis of the *exo*-isomer of compound **1** was adapted from a published approach[1]: A round bottom flask containing 20 g (204 mmol) of maleic anhydride was charged with 160 mL of toluene, stirred for 30 minutes and filtered. 16.64 g (244 mmol) of furan was added to the filtrate and stirred for 48 hours at room temperature. The precipitate was collected, filtered, and washed with cold toluene to yield a white solid (22.03 g, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.58 (2 H, s, -CHC*H*=C*H*CH-), 5.46 (2 H, s, -C*H*CH=CHC*H*-), 3.17 (2 H, s, O=CC*H*).

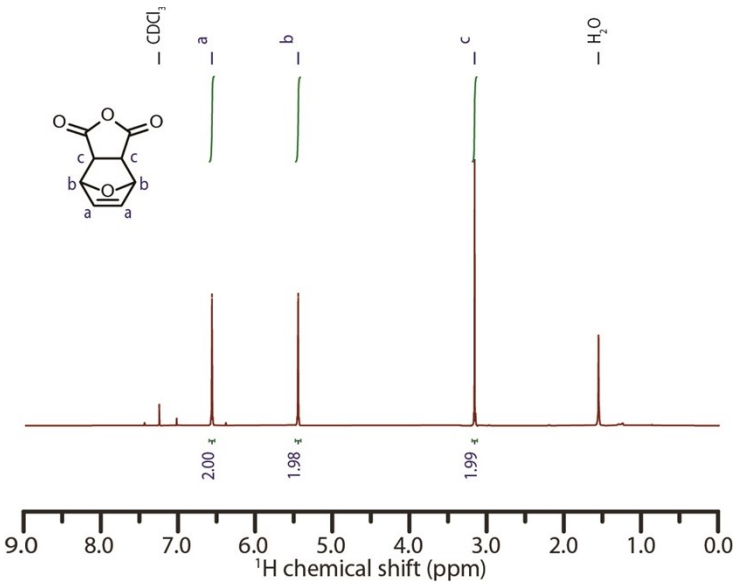


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$) of compound **1**.

Synthesis of compound **2:** Compound **1** (10 g, 60 mmol), ethanol (30 mL), and triethylamine (TEA, 10 mL) were placed in a 250 mL round bottom flask equipped with a magnetic stirrer. To this, a solution of *tert*-butyl-2-aminoethyl carbamate (10.12 g, 63.1 mmol) and 20 mL ethanol were added dropwise with continuous stirring, and mixed for one hour. The resulting mixture was refluxed at 85°C, using a reflux condenser, for 4 hours. The solution was cooled to room temperature overnight and the white solid was filtered off and recrystallized from ethanol. The collected crystals were dried under vacuum to afford 2 (9.39 g, 51% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.51 (2 H, s, -CHC*H*=C*H*CH-), 5.26 (2 H, s, -C*H*CH=CHC*H*-), 3.63 (2 H, t, NCH_2CH_2NHC=O), 3.31 (2 H, t, NCH_2CH_2NHC=O), 2.85 (2 H, s, O=CC*H*), 1.41 (9 H, t, C(CH_3)$_3$).

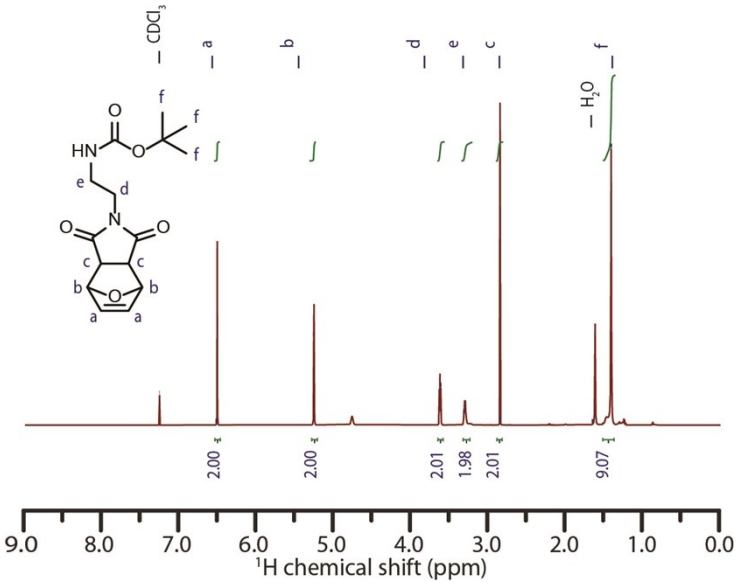


Figure S2. ^{1}H NMR (400 MHz, $CDCl_3$) of compound **2**.

Synthesis of compound **3,** Nfpm: In a 500 mL round bottom flask, compound **2** (9.4 g, 30.5 mmol) was dissolved in a 3:7 mixture of trifluoroacetic acid (TFA)/dichloromethane (DCM) and stirred at room temperature for one hour before blowing off with a gentle stream of nitrogen and subsequent solvent removal by evaporation. The resulting oil was added to diethyl ether to

precipitate. After centrifugation, the supernatant was discarded and diethyl ether was again added to wash the product. This was repeated three times before collecting and drying the product under vacuum, quantitatively yielding compound **3** as a white solid. ^{1}H NMR (400 MHz, D_2O) δ: 6.65 (2 H, s, -CHC*H*=C*H*CH-), 5.37 (2 H, s, -C*H*CH=CHC*H*-), 3.86 (2 H, t, NCH_2CH_2NH_2), 3.31 (2 H, t, NCH_2CH_2NH_2), 3.21 (2 H, s, O=CC*H*).

Oligomer synthesis

The peptoid-based oligomers were prepared using a microwave-assisted Liberty Blue automated peptide synthesizer (CEM Corporation) programmed with a peptoid synthetic method. Peptoids were synthesized on acid-labile Rink amide resin (ChemPep) bearing a fluorenylmethyloxycarbonyl (Fmoc)-protected amine that is initially deprotected prior to synthesis by treatment in 4-methylpiperidine:dimethylformamide (DMF) (20:80, volume ratio) to yield a terminal amine on the solid support. The synthesis then proceeds by sequential addition reactions whereby a terminal amine is acetylated with 1 M bromoacetic acid using 1.2 M diisopropylcarbodiimide (DIC) as an activator for 5 minutes at 75°C, to afford a terminal bromide which is subsequently displaced via nucleophilic substitution with a 0.5 M primary amine for 5 minutes at 75°C. This two-step process is followed to synthesize the different predefined sequences. Notably, addition of Nfpm, the furan-protected maleimide-bearing primary amine monomer, additionally requires 1.3 equivalents of *N,N*-diisopropylethylamine (DIPEA) to deprotonate the primary amine during the nucleophilic substitution. The *N*-terminal of the complementary oligomers was capped with 1 M acetic anhydride activated with DIC to prevent further chain elongation. The primary amines fall into two categories, dynamic covalent functional groups and inert spacer monomers. The dynamic covalent functional group consisted of the prepared Nfpm and commercially available furfuryl amine, Nfur. The inert spacer monomers were the commercially available 2-methoxyethylamine (Nmea) and 2-ethoxyethoxyethylamine (Neee) prepared according to the established protocol of Wei et al.[2] All of the reagents were prepared in dimethylformamide (DMF).

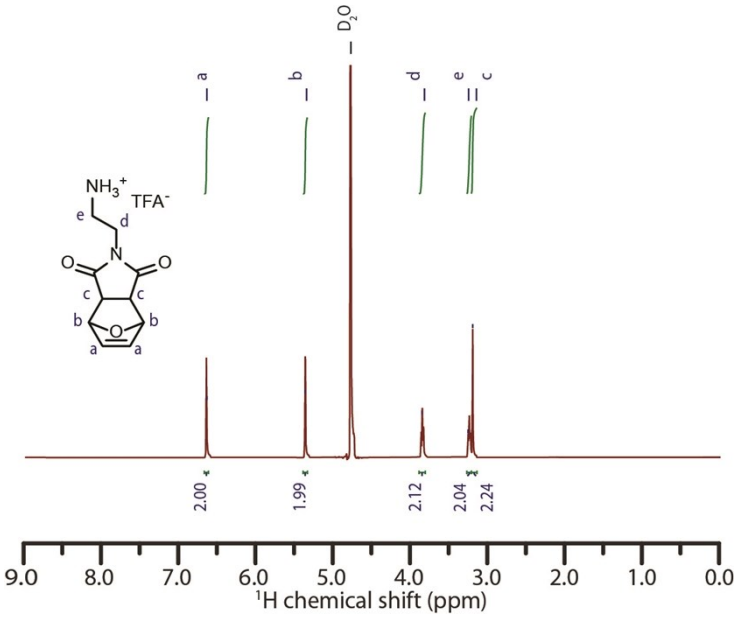
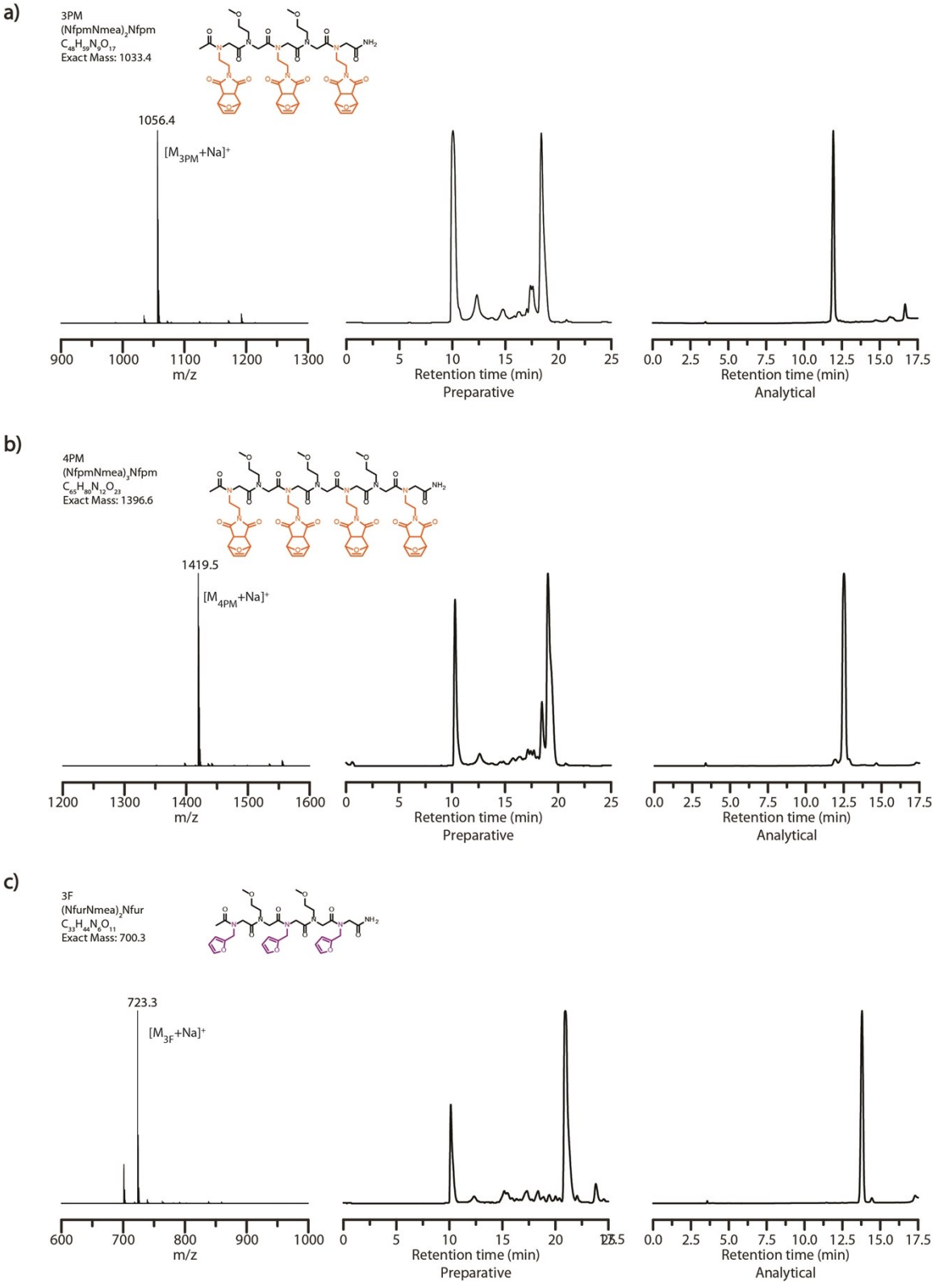


Figure S3. ^{1}H NMR (400 MHz, D_2O) of compound **3**.

Oligomer cleavage and purification

Peptoids were cleaved from acid-labile Rink amide resin by a 10 minute incubation with a cleavage cocktail containing 95% TFA and 5% water in a glass fritted reaction vessel. The resin was then rinsed with TFA to remove any residual peptoid on the resin. TFA and trace water were removed by blowing with a N_2 stream. Once only the peptoid residue remained, the residue was dissolved in a 50:50 solution of acetonitrile and water and purified by preparative scale reverse phase-HPLC using a linear gradient of acetonitrile and water. Major peaks were collected and characterized by ESI mass spectrometry to confirm the identity of each strand. Fractions containing the peptoids were lyophilized to a white powder.


a)
3PM
(NfpmNmea)2Nfpm
C48H59N9O17
Exact Mass: 1033.4
1056.4
[M3PM+Na]+
900 1000 1100 1200 1300
m/z
0 5 10 15 20 25
Retention time (min)
Preparative
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5
Retention time (min)
Analytical
b)
4PM
(NfpmNmea)3Nfpm
C65H80N12O23
Exact Mass: 1396.6
1419.5
[M4PM+Na]+
1200 1300 1400 1500 1600
m/z
0 5 10 15 20 25
Retention time (min)
Preparative
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5
Retention time (min)
Analytical
c)
3F
(NfurNmea)2Nfur
C33H44N6O11
Exact Mass: 700.3
723.3
[M3F+Na]+
600 700 800 900 1000
m/z
0 5 10 15 20
Retention time (min)
Preparative
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5
Retention time (min)
Analytical

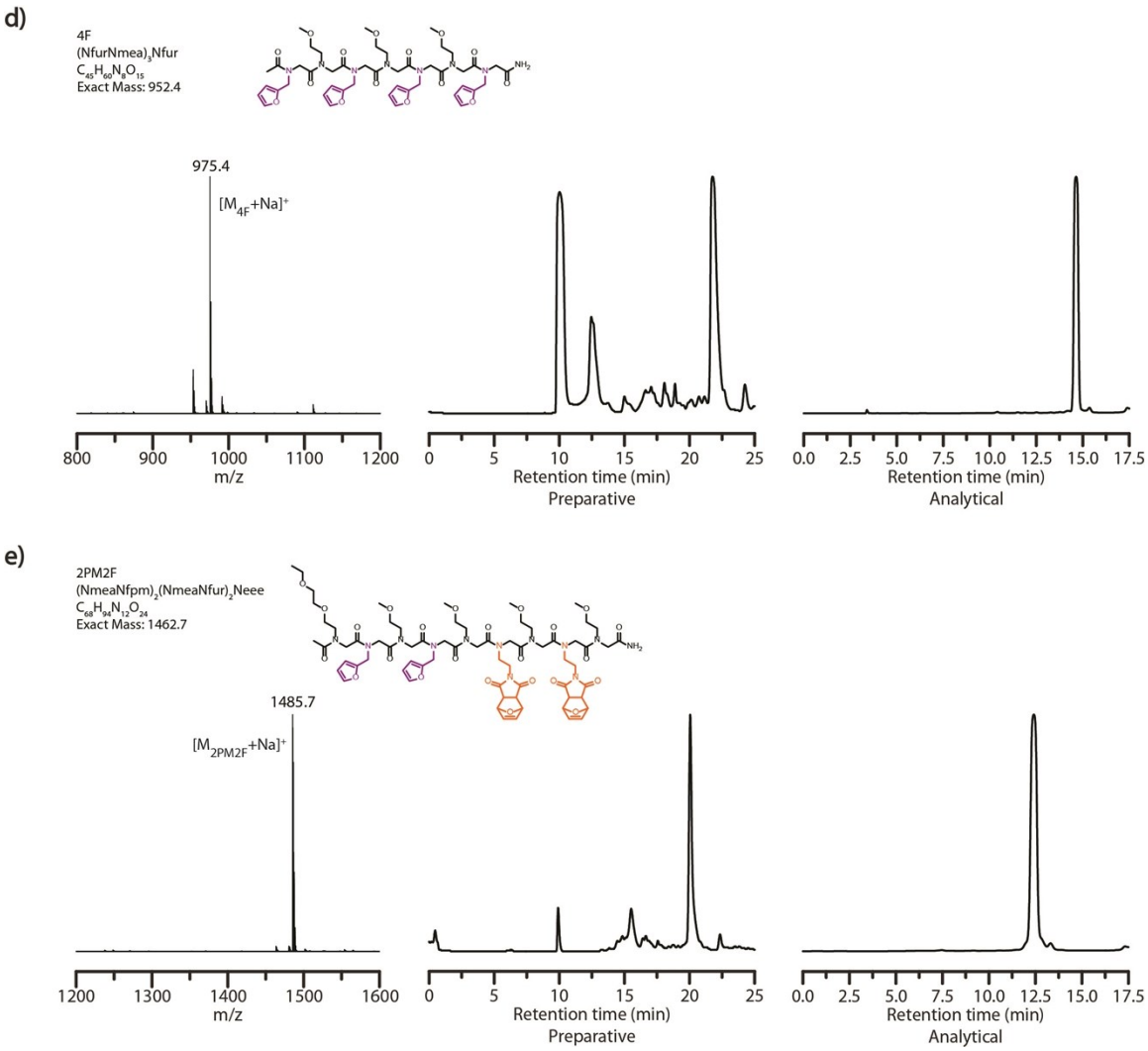


Figure S4. ESI mass spectra, preparative HPLC traces, and corresponding analytical HPLC traces of peptoids synthesized for this study.

Oligomer deprotection

Peptoids were resuspended in acetonitrile to afford 10 mM stock solutions. 20 µL of furan-protected maleimide-bearing oligomers was added to 100 µL of anhydrous anisole and stirred at 140°C, or other specified temperature, in a vial without a cap for 30 minutes such that the adduct undergoes the retro-Diels-Alder reaction. For HPLC and NMR analysis of deprotected solutions, mixtures were subjected to vacuum evaporation under reduced pressure and resuspended in deuterated acetonitrile.

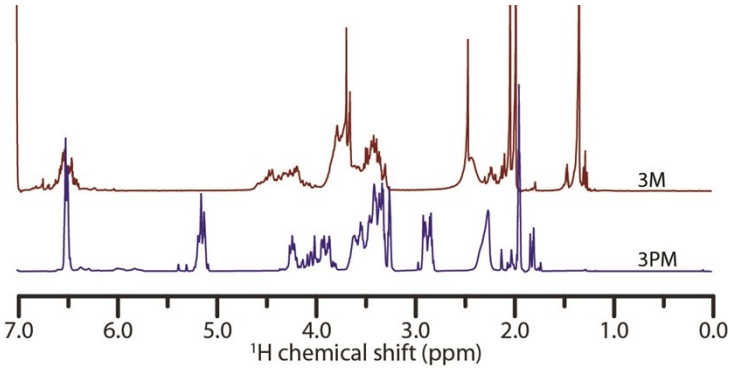


Figure S5. ^{1}H NMR (400 MHz, CD_3CN) of a trifunctional peptoid bearing maleimide species before (bottom, blue) and after deprotection (top, red).

Molecular ladder formation

Dissociation and deprotection of peptoids was performed by adding 20 µL from a 10 mM stock solution of each desired oligo(peptoid) sequence to a 100 µL solution of anhydrous anisole which was stirred at 140°C for 30 minutes in a vial without a cap prior to cooling either 60°C or 100°C and addition of a further 200 µL of anhydrous anisole. TEA was often added to reaction solutions and stirred for 15 minutes before analysis ESI-MS to increase ionization efficiency of ladder species.

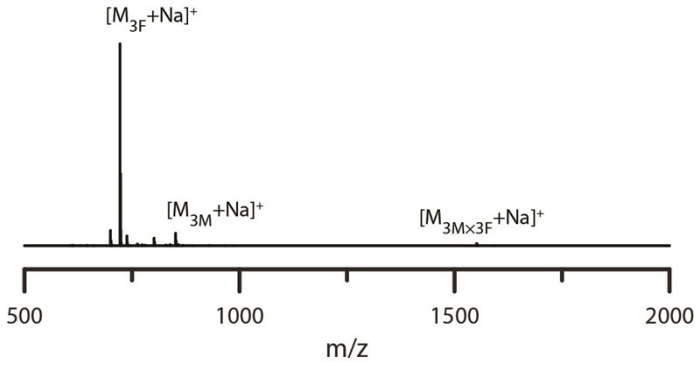


Figure S6. Mass spectra of a 3M×3F hybridization solution after five days at 60°C and absent any catalyst.

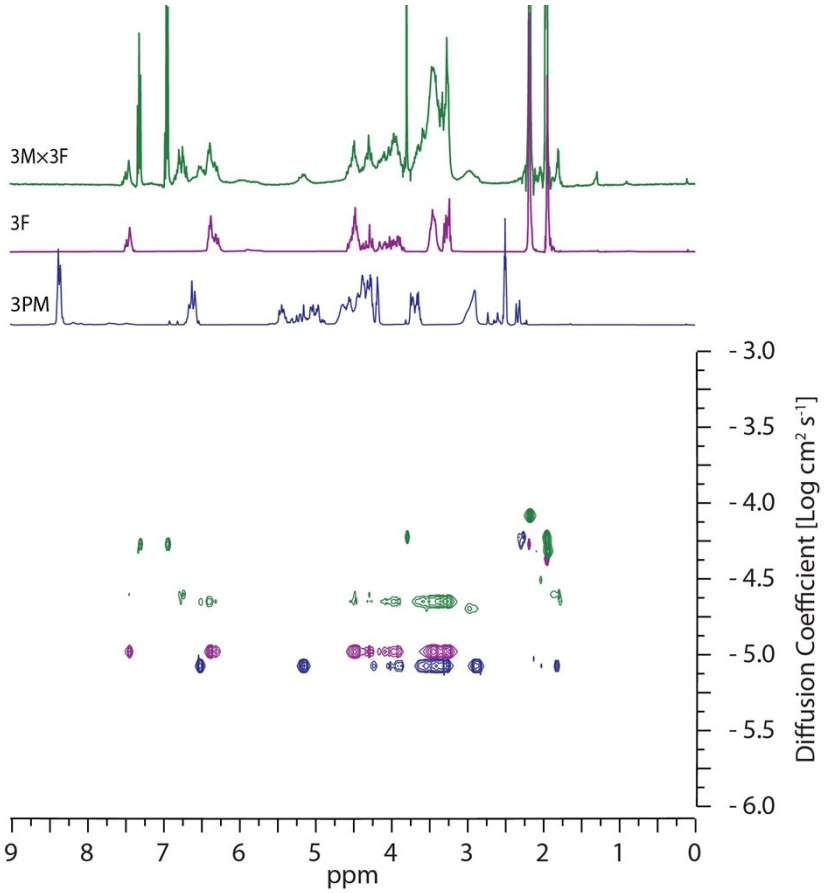


Figure S7. DOSY NMR spectra of 3PM (bottom, blue), 3F (middle, purple) and a 3M×3F molecular ladder mixture (top, green) after reaction for two days at 60°C and absent any catalyst. As hybridization reactions in deuterated anisole generated significant amounts of precipitate, CD_3CN was used as the solvent for DOSY NMR studies.

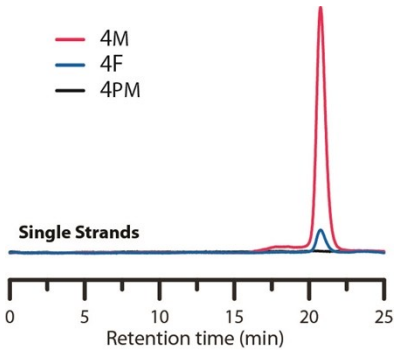


Figure S8. GPC traces of sequence-defined oligomers using a UV-Vis detector at 313 nm.

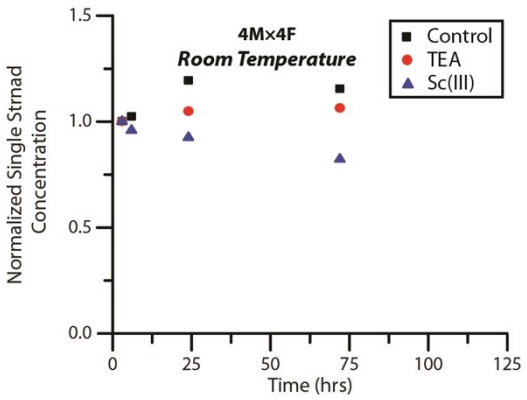


Figure S9. Normalized concentrations of single strand species in hybridization mixtures with and without added catalysts at room temperature found by deconvoluting GPC traces with fitted Gaussian functions and normalized to an internal polystyrene standard.

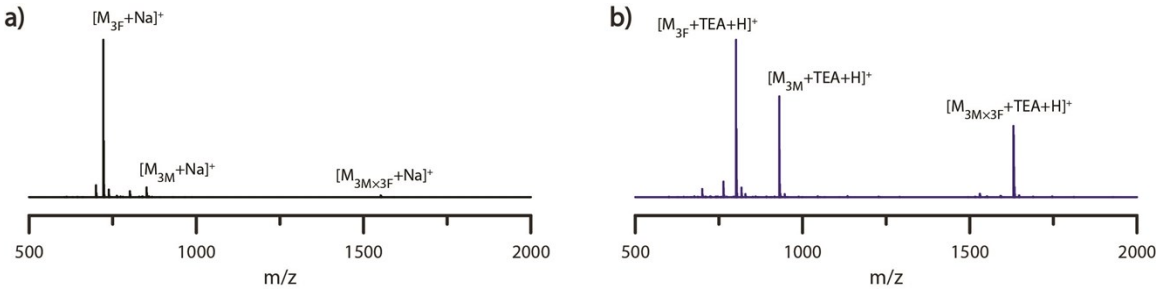


Figure S10. ESI mass spectra of a 3M×3F hybridization mixture at 60°C **(a)** before and **(b)** 15 minutes after addition of a catalytic amount of TEA. Expected exact masses: $[M_{3F}+Na]^+$ = 723.3; $[M_{3M}+Na]^+$ = 852.3; $[M_{3M\times3F}+Na]^+$ = 1552.6; $[M_{3F}+TEA+H]^+$ = 802.4; $[M_{3M}+TEA+H]^+$ = 931.4; $[M_{3M\times3F}+TEA+H]^+$ = 1631.7. Exact masses found: $[M_{3F}+Na]^+$ = 723.3; $[M_{3M}+Na]^+$ = 852.3; $[M_{3M\times3F}+Na]^+$ = 1552.6; $[M_{3F}+TEA+H]^+$ = 802.4; $[M_{3M}+TEA+H]^+$ = 931.5; $[M_{3M\times3F}+TEA+H]^+$ = 1631.8.

Molecular ladder degree of alignment analysis

To single strand and hybridization mixtures, 5 µL of TEA and various equivalents of methyl 3-mercaptopropionate (S) per maleimide moiety were added and stirred for 30 minutes prior to analysis. For HPLC and NMR analysis of deprotected solutions, mixtures were evaporated under reduced pressure and resuspended in deuterated acetonitrile.

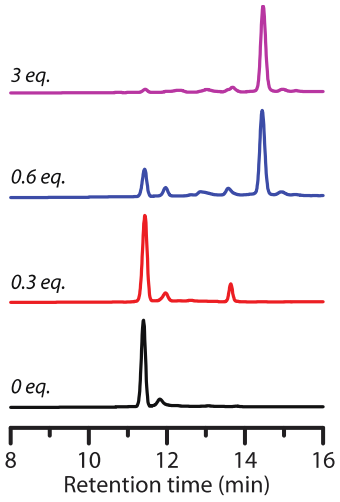


Figure S11. HPLC traces generated from thiol-Michael addition reaction mixtures between the peptoid, 3M, and various equivalents of S to maleimide groups.

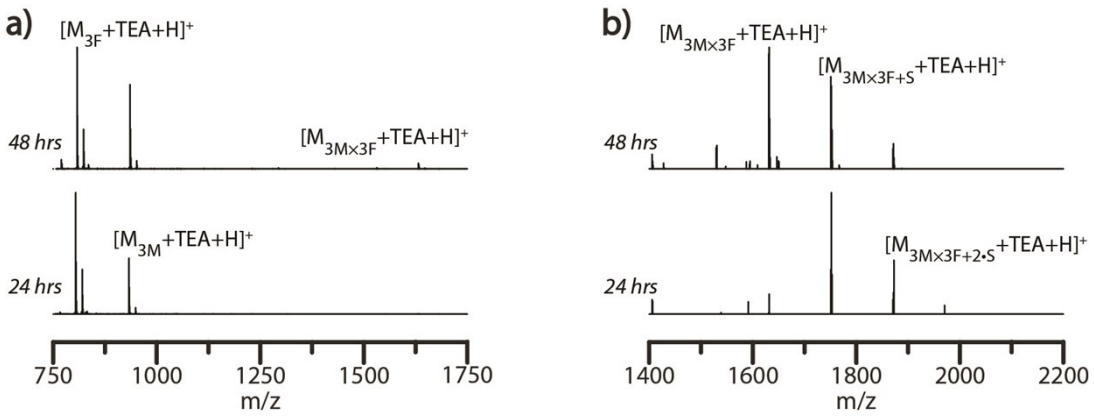


Figure S12. ESI mass spectra of 3M×3F hybridization mixtures **(a)** after reaction at 100°C for 24 and 48 hours, and **(b)** after reaction at 100°C for 24 and 48 hours, then subjected to a thiol-Michael addition with a threefold excess of S. Expected exact masses: $[M_{3F}+TEA+H]^+$ = 802.4; $[M_{3M}+TEA+H]^+$ = 931.4; $[M_{3M\times3F}+TEA+H]^+$ = 1631.7; $[M_{3M+S}+TEA+H]^+$ = 1051.4; $[M_{3M+2\bullet S}+TEA+H]^+$ = 1171.3; $[M_{3M+3\bullet S}+TEA+H]^+$ = 1291.3; $[M_{3M\times3F+S}+TEA+H]^+$ = 1751.7; $[M_{3M\times3F+2\bullet S}+TEA+H]^+$ = 1871.7;. Exact masses found: $[M_{3F}+TEA+H]^+$ = 802.4; $[M_{3M}+TEA+H]^+$ = 931.4; $[M_{3M\times3F}+TEA+H]^+$ = 1631.7; $[M_{3M+S}+TEA+H]^+$ = 1051.5; $[M_{3M+2\bullet S}+TEA+H]^+$ = 1171.5; $[M_{3M+3\bullet S}+TEA+H]^+$ = 1291.5; $[M_{3M\times3F+S}+TEA+H]^+$ = 1751.8; $[M_{3M\times3F+2\bullet S}+TEA+H]^+$ = 1871.8.

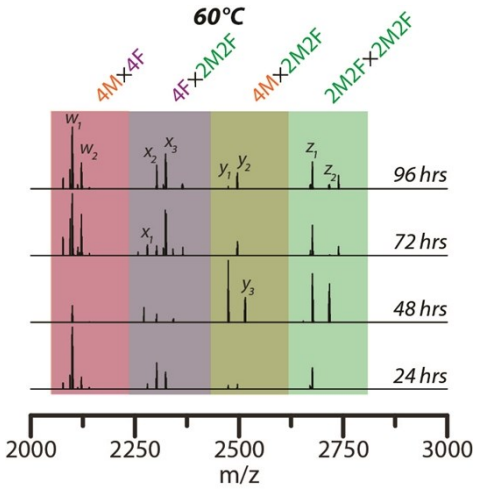


Figure S13. Dynamic covalent assembly of sequence-defined molecular ladders. ESI mass spectra of molecular ladder reaction mixture aliquots removed from hybridization solutions maintained at 60°C. Each time point shown indicates the period at 60°C. Expected exact masses: w_1 = $[M_{4M\times4F}+Na]^+$ = 2099.8; w_2 = $[M_{4M\times4F}+2Na-H]^+$ = 2121.8; x_1 = $[M_{4F\times2M2F}+H]^+$ = 2280.0; x_2 = $[M_{4F\times2M2F}+Na]^+$ = 2303.0; x_3 = $[M_{4F\times2M2F}+2Na-H]^+$ = 2324.0; y_1 = $[M_{4M\times2M2F}+Na]^+$ = 2474.0; y_2 = $[M_{4M\times2M2F}+2Na-H]^+$ = 2496.0; y_3 = $[M_{4M\times2M2F}+MeCN+Na]^+$ = 2515.1; z_1 = $[M_{2M2F\times2M2F}+Na]^+$ = 2676.2; z_2 = $[M_{2M2F\times2M2F}+MeCN+Na]^+$ = 2717.2. Exact masses found: w_1 = $[M_{4M\times4F}+Na]^+$ = 2099.8; w_2 = $[M_{4M\times4F}+2Na-H]^+$ = 2121.8; x_1 = $[M_{4F\times2M2F}+H]^+$ = 2280.0; x_2 = $[M_{4F\times2M2F}+Na]^+$ = 2303.0; x_3 = $[M_{4F\times2M2F}+2Na-H]^+$ = 2324.0; y_1 = $[M_{4M\times2M2F}+Na]^+$ = 2474.0; y_2 = $[M_{4M\times2M2F}+2Na-H]^+$ = 2496.0; y_3 = $[M_{4M\times2M2F}+MeCN+Na]^+$ = 2515.0; z_1 = $[M_{2M2F\times2M2F}+Na]^+$ = 2676.2; z_2 = $[M_{2M2F\times2M2F}+MeCN+Na]^+$ = 2717.1.

References

1 S. R. Mane, V. Rao N, K. Chatterjee, H. Dinda, S. Nag, A. Kishore, J. Das Sarma and R. Shunmugam, *J. Mater. Chem.*, 2012, **22**, 19639–19642.

2 T. Wei, J. H. Jung and T. F. Scott, *J. Am. Chem. Soc.*, 2015, **137**, 16196–16202.